



## Using Diquat in Combination with Endothall Under Turbid Water Conditions to Control Hydrilla

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**PURPOSE:** This small-scale study examined three turbidity levels in the water column to investigate the efficacy of diquat (6,7-dihydrodipyrido[1,2- $\alpha$ :2',1'- $c$ ]pyrazinedium ion) in combination with the dipotassium salt of endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) to control hydrilla (*Hydrilla verticillata* (L.f.) Royle).

**BACKGROUND:** Diquat is an effective contact herbicide for controlling noxious aquatic weeds. For controlling submersed weeds, it is typically used in small treatment areas near boat docks, swimming beaches, and lake access points. Frequently, it is applied in areas where there is rapid water exchange as it quickly interrupts plant photosynthesis causing plant death in 4 to 7 days. Symptoms may be evident in as little as a few hours after treatment. Details of the diquat mode of action are reviewed by Hess (2000).

Turbid water may interfere with the efficacy of diquat treatments (Bowmer 1982a, 1982b). Turbidity is caused by suspended sediments that are largely composed of negatively charged clay particles. Because diquat is a cationic herbicide, it is strongly adsorbed by these clays, potentially rendering it unavailable for plant uptake. Adsorption of diquat is dependent upon amount and type of clay present in the water column (Weber et al. 1965, Narine and Guy 1982).

Poovey and Getsinger (2002) reported the effect of turbidity from two different clay types on the bioavailability of diquat against egeria (*Egeria densa* Planch.). Turbidity caused by a 100-percent bentonite clay sediment prevented the bioavailability of diquat at turbidity levels greater than 5 nephelometric turbidity units (NTU). Diquat uptake was not inhibited by 20-percent montmorillonite clay sediment; egeria was controlled with diquat cation rates of 0.7 to 1.5 mg ai L<sup>-1</sup> at 15 NTU.

Turbidity from a 20-percent kaolinite clay sediment interfered with diquat efficacy for control of coontail (*Ceratophyllum demersum* L.) in a greenhouse study (Hofstra et al. 2001). Although 2 mg ai L<sup>-1</sup> diquat cation provided 60 to 70 percent control with water column turbidities of 10 and 25 NTU, respectively, the authors concluded that the effect was not significant because firm and intact stems were observed after treatment, which signified potential plant recovery.

Failure to achieve efficacy against submersed aquatic weeds in the field may be due to turbidity in treatment areas. Reduced control was noted in turbid water using a low dose of diquat (0.13 mg L<sup>-1</sup> diquat cation) in combination with a low dose of endothall (1.0 mg L<sup>-1</sup>) to control hydrilla on Toledo Bend Reservoir, Louisiana-Texas in 2000 (Skogerboe, unpublished data). These rates provided 95 to 100 percent control of hydrilla in a small-scale greenhouse study (Pennington et al. 2001) and in the same plots when no turbidity was observed (Skogerboe et al. 2004).

**MATERIALS AND METHODS:** This study was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS in a controlled environment growth chamber. The chamber contained 24 vertical aquaria (50-L capacity) that were plumbed to conduct flow-through exposures with continuous air circulation, which provided complete mixing of water. During this study, the photoperiod was 14L:10D, with a light intensity of  $575 \pm 50 \text{ } \mu\text{mol m}^{-2} \text{ sec}^{-1}$ , and a temperature of  $21 \pm 2 \text{ C}$ .

Sediment from Brown's Lake, Vicksburg, MS was amended with  $150 \text{ g L}^{-1}$  ammonium chloride to provide adequate nutrients for plant growth. The sediment was then put in 1-L plastic containers. Five healthy apices (15 cm) of dioecious hydrilla (obtained from a pond at Lewisville Aquatic Ecosystem Research Facility-ERDC, Lewisville, TX) were planted in each container. Sand capped the sediment surface to prevent the sediment from mixing with the water column. Four containers were placed in each vertical aquarium filled with culture solution (Smart and Barko 1985). Plants grew to form a lush surface canopy in four weeks.

Using a field-collected sediment from Toledo Bend Reservoir (LA-TX), three turbidity levels (0, 10, and 20 NTU) were used to evaluate two rates of diquat, as Reward®,<sup>1</sup> 0.18, and  $0.36 \text{ mg ai L}^{-1}$ , calculated as the cation, used in combination with  $1.0 \text{ mg ai L}^{-1}$  endothall, as Aquathol® K,<sup>1</sup> for a 24-hr exposure time. The maximum label rate for Reward is  $0.36 \text{ mg ai L}^{-1}$  while the maximum label rate for Aquathol K is  $5.0 \text{ mg ai L}^{-1}$ . In addition, there were  $0 \text{ mg ai L}^{-1}$  diquat/0 NTU (reference) and  $0 \text{ mg ai L}^{-1}$  diquat/20 NTU treatments for comparison of plant status in the absence of herbicide treatment.

The Toledo Bend sediment was collected in Diamond Cove, a bay on the Louisiana side of the reservoir, where herbicide applications using diquat and endothall had taken place in September 2000. Collected sediment was analyzed by the ERDC Geotechnical and Structures Laboratory (C. A. Weiss). X-ray diffraction analysis ( $<2\mu$ ) showed that this sediment contained about 17 percent clay that was a mix of montmorillonite  $[(\text{Al},\text{Mg})_2\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}]$  and kaolinite  $[\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4]$  clays. The cation exchange capacity (CEC) was  $3 \text{ meq 100 g}^{-1}$ .

Turbidity of 10 and 20 NTU was created in the water column by mixing 15 and 30 g of sediment, respectively, with 500 ml water to create a slurry (Figure 1). These slurries were stirred continuously for 24 hr, then poured into each treatment aquarium 4 hr before herbicide application. Some sediment particles also coated plant stems and leaves. Stock solutions of diquat ( $4.8 \text{ mg ai L}^{-1}$ ) as Reward®<sup>1</sup> (Syngenta Professional Products, Greensboro, NC) and endothall ( $2.5 \text{ g ai L}^{-1}$ ) as Aquathol® K<sup>1</sup> (Cerexagri, King of Prussia, PA), were applied to aquaria to achieve target treatment concentrations. During herbicide exposure, turbid conditions were maintained by vigorously bubbling air into the water column augmented with occasional gentle stirring with a meter stick. Turbidity was monitored pretreatment, and 3, 18, and 24 hr after treatment (HAT) with a portable turbidimeter (Hach Model 2100P). After 24 hr, peristaltic pumps evacuated the treated turbid water and fresh clear water was pumped into the aquaria, except for the  $0 \text{ mg ai L}^{-1}$  diquat/20 NTU treatments. Turbidity in the  $0 \text{ mg ai L}^{-1}$  diquat/0 NTU and  $0 \text{ mg ai L}^{-1}$  diquat/20 NTU replicates was maintained through the duration of the study.

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<sup>1</sup> Mention of trade names is not intended to recommend one product over another.

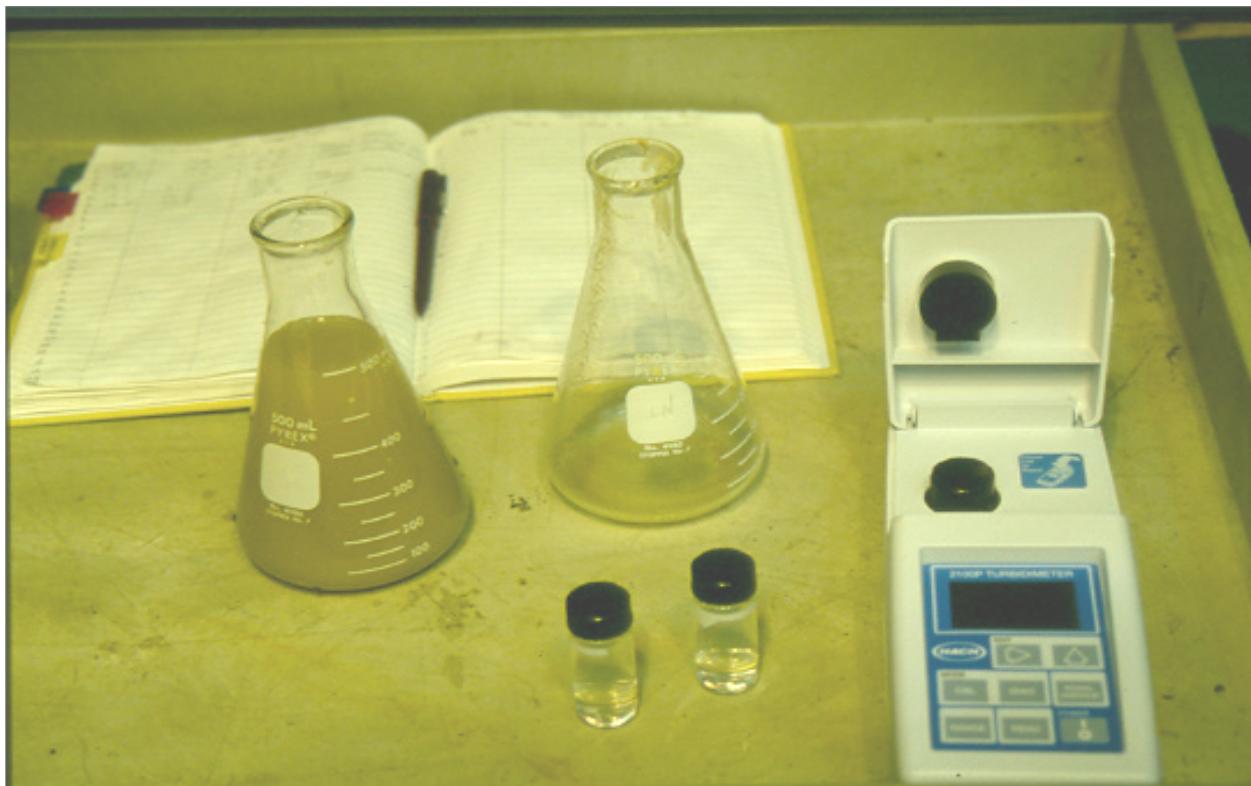


Figure 1. Slurries made from mixing water with sediment collected from Toledo Bend Reservoir, LA-TX. These slurries were applied to the water column 4 hr before herbicide treatment. Turbidity in the water column was measured with a HACH Turbidimeter 2100P

Tissue samples (0.1 to 0.2 g) were removed from plant apices in each aquarium at 1, 3, and 6 weeks after treatment (WAT) and analyzed for total chlorophyll content (Hiscox and Israelstam 1979) using 10-mL dimethylsulfoxide (DMSO) for extraction. Leaf chlorophyll content provided an assessment of the general health of a plant following herbicide treatment and is reported as mg chlorophyll  $\text{g}^{-1}$  plant fresh weight.

At 3 and 6 WAT, two containers were removed from each aquarium, shoot biomass was collected and dried for 48 hr at 70 °C, then weighed. Shoot biomass is reported as g dry weight (DW).

Treatments were assigned to individual aquaria in a completely randomized manner and replicated three times. Biomass and chlorophyll data were subjected to a two-way analysis of variance (ANOVA) to determine the herbicide and turbidity effects. If statistical differences occurred between treatments, means were separated using the Tukey test ( $p \leq 0.05$ ).

**RESULTS AND DISCUSSION:** Turbidity measurements in treatment aquaria during herbicide exposure are shown in Figure 2. Turbidity was below 1 NTU in all treatments with a target of 0 NTU. The 10 NTU treatments ranged from 8 to 14 NTU. Although the 20 NTU treatments varied from 11 to 33 NTU, the mean ( $\pm 1$  SE) for both herbicide combination treatments was  $19.90 \pm 1.4$  NTU. Throughout the study, turbidity was maintained in aquaria not treated with herbicides (mean =  $22.79 \pm 7.23$ ,  $n = 76$ ). This treatment was similar to the untreated reference (0 mg ai  $\text{L}^{-1}$ )

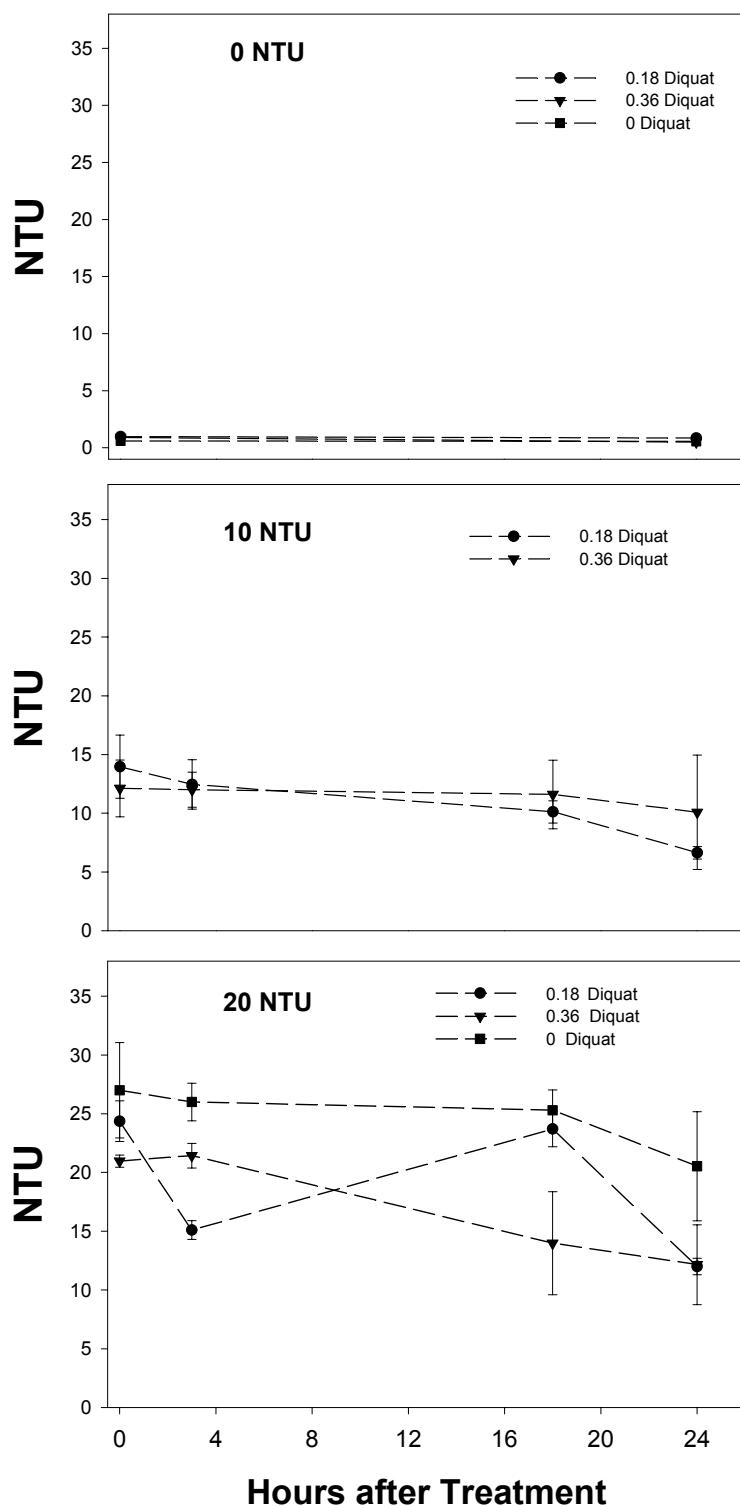


Figure 2. Turbidity (NTU) in treatment aquaria during herbicide exposure (24 hr). Measurements (mean  $\pm 1$  SE) were taken pretreatment, 3, 18, and 24 hr after herbicide application of diquat (0, 0.18, or 0.36 mg ai L<sup>-1</sup>) and endothall (1.0 mg ai L<sup>-1</sup>). The 0 diquat application at 0 NTU was not combined with endothall and served as a reference

diquat/0 mg ai L<sup>-1</sup> endothall /0 NTU treatment). There were no significant differences in biomass (Figure 3) nor leaf chlorophyll concentrations (Table 1), indicating that turbidity levels used in this study without herbicide application did not affect hydrilla growth.

Herbicide application without turbidity reduced hydrilla biomass both 3 and 6 WAT (Figure 3). Both combinations of diquat with endothall were effective in reducing shoot biomass by more than 90 percent at 3 WAT and more than 99 percent at 6 WAT. When turbidity was added to the water column, however, efficacy of diquat in combination with endothall varied with treatment rate and turbidity level. At 10 and 20 NTU, the 0.18 mg ai L<sup>-1</sup> diquat/1 mg ai L<sup>-1</sup> endothall combination was similar to the reference both 3 and 6 WAT (Figure 3). Although lowered leaf chlorophyll concentrations suggested that this combination injured the plant at 10 NTU, injury was not evident at 20 NTU (Table 1). At 10 NTU, the 0.36 mg ai L<sup>-1</sup> diquat/1 mg ai L<sup>-1</sup> endothall combination significantly decreased hydrilla biomass by 80 percent at 3 WAT and 90 percent at 6 WAT (Figure 3). This treatment also severely injured the plant's physiological processes causing depressed leaf chlorophyll concentrations early in the study (Table 1). Accordingly, there were no apices to sample because plant biomass was decaying by 6 WAT. At 20 NTU, leaf chlorophyll concentrations indicated that the 0.36 mg ai L<sup>-1</sup> diquat/1 mg ai L<sup>-1</sup> endothall combination affected plant physiology by 7 WAT (Table 1); however, hydrilla continued to accumulate biomass throughout the study (Figure 3).

Results of this small-scale study indicated that low concentrations of diquat in combination with low concentrations of endothall did not control hydrilla at turbidity levels of 10 to 20 NTU. A higher rate of diquat in combination with endothall was more effective in suppressing hydrilla biomass. It is uncertain whether this suppression was due to the combination of the two products or the effect of endothall alone. In an outdoor mesocosm study, using a 24-hr half-life exposure, 1.0 mg ai L<sup>-1</sup> endothall reportedly controlled hydrilla by 80 percent when used alone (Skogerboe and Getsinger 2001). Although control of hydrilla may increase by more than 20 percent when low rates of endothall are combined with diquat (Pennington et al. 2001), endothall is not adversely affected by turbidity (Hofstra et al. 2001).

**FUTURE WORK:** Further research on different combinations of diquat and endothall will elucidate how to use diquat more effectively for hydrilla control in turbid treatment areas.

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Poovey, A. G., and Skogerboe, J.G. (2003). "Using diquat in combination with endothall under turbid conditions to control hydrilla," *APCRP Technical Notes Collection* (ERDC/TN APCRP-CC-02), U.S. Army Engineer Research and Development Center, Vicksburg, MS. [www.wes.army.mil/el/aqua](http://www.wes.army.mil/el/aqua).

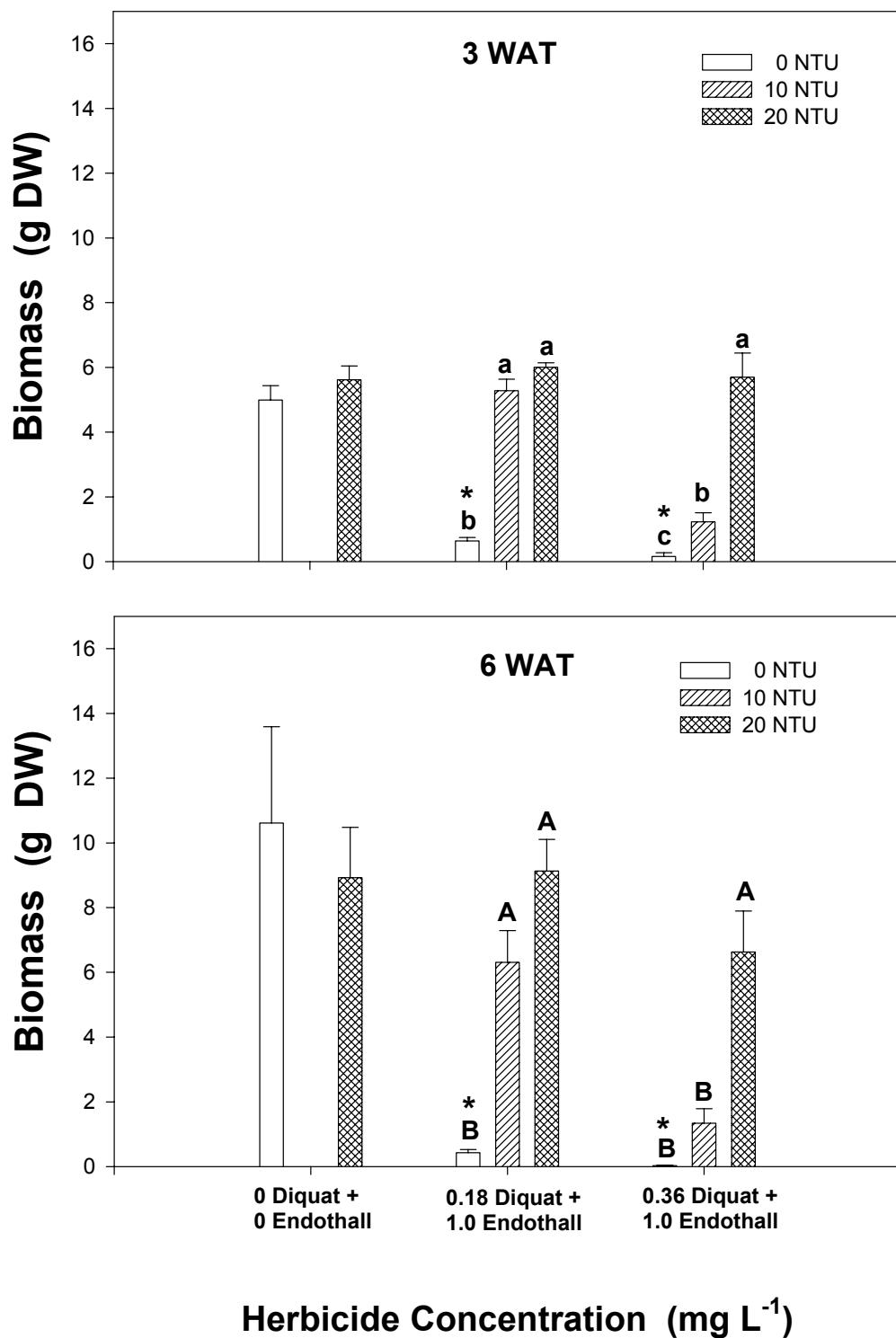


Figure 3. Hydrilla shoot biomass (g DW) 3 and 6 weeks after treatment (WAT). Plants were treated with diquat (0, 0.18, or 0.36 mg ai L<sup>-1</sup>) in combination with endothall (0 or 1 mg ai L<sup>-1</sup>) under turbid conditions (0, 10, and 20 NTU). Means are ±1 SE (n = 3); different letters denote significant differences between levels of turbidity for a herbicide concentration (Tukey test, p ≤ 0.05)

**Table 1**  
**Chlorophyll Concentrations ( $\text{mg g}^{-1}$  FW) in *Hydrilla* Apices 1, 3 and 6 Weeks After Treatment (WAT)<sup>1</sup>**

Turbidity Level	Chlorophyll, $\text{mg g}^{-1}$ FW		
	1 WAT	3 WAT	6 WAT
<b>0 diquat + 0 endothall</b>			
0 NTU	1.03 ± 0.12	1.00 ± 0.02	0.88 ± 0.08
20 NTU	0.91 ± 0.10	0.82 ± 0.03	0.87 ± 0.01
<b>0.18 diquat + 1.0 endothall</b>			
0 NTU	0.03 ± 0.01 c	—	—
10 NTU	0.69 ± 0.03 b	0.56 ± 0.14 b	0.48 ± 0.24 b
20 NTU	0.75 ± 0.08 a	0.89 ± 0.02 a	1.04 ± 0.15 a
<b>0.36 diquat + 1.0 endothall</b>			
0 NTU	0.24 ± 0.03	—	—
10 NTU	0.27 ± 0.07	0.20 ± 0.10	—
20 NTU	0.48 ± 0.07	0.31 ± 0.05	0.41 ± 0.21

<sup>1</sup> Plants were treated with diquat (0, 0.18, or 0.36 mg ai L<sup>-1</sup>) in combination with endothall (0 or 1 mg ai L<sup>-1</sup>) under turbid conditions (0, 10, and 20 NTU). Means are ±1 SE (n = 3); different letters in a column denote significant differences between levels of turbidity for a herbicide concentration (Tukey test ≤ 0.05). A dash indicates there were no apices for collection.

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